CLINICAL UTILITY AND INTERPRETATION OF DIAGNOSTIC TESTS FOR BOVINE RESPIRATORY DISEASE

BRENT CREDILLE, DVM, PHD, DACVIM
ASSISTANT PROFESSOR
SECTION HEAD – FARM PRACTICE, THERIOGENOLOGY, AND PRODUCTION MEDICINE
DIRECTOR, FOOD ANIMAL HEALTH AND MANAGEMENT PROGRAM
DEPARTMENT OF POPULATION HEALTH
COLLEGE OF VETERINARY MEDICINE
UNIVERSITY OF GEORGIA

OVERVIEW

Bovine Respiratory Disease

- **Introduction**

- **Epidemiology**

Diagnostic Tests

- **Terminology and Characteristics**

Availability of Tests for BRD Pathogens

- **Advantages and Disadvantages**

- **Recommendations for Use**

BOVINE RESPIRATORY DISEASE

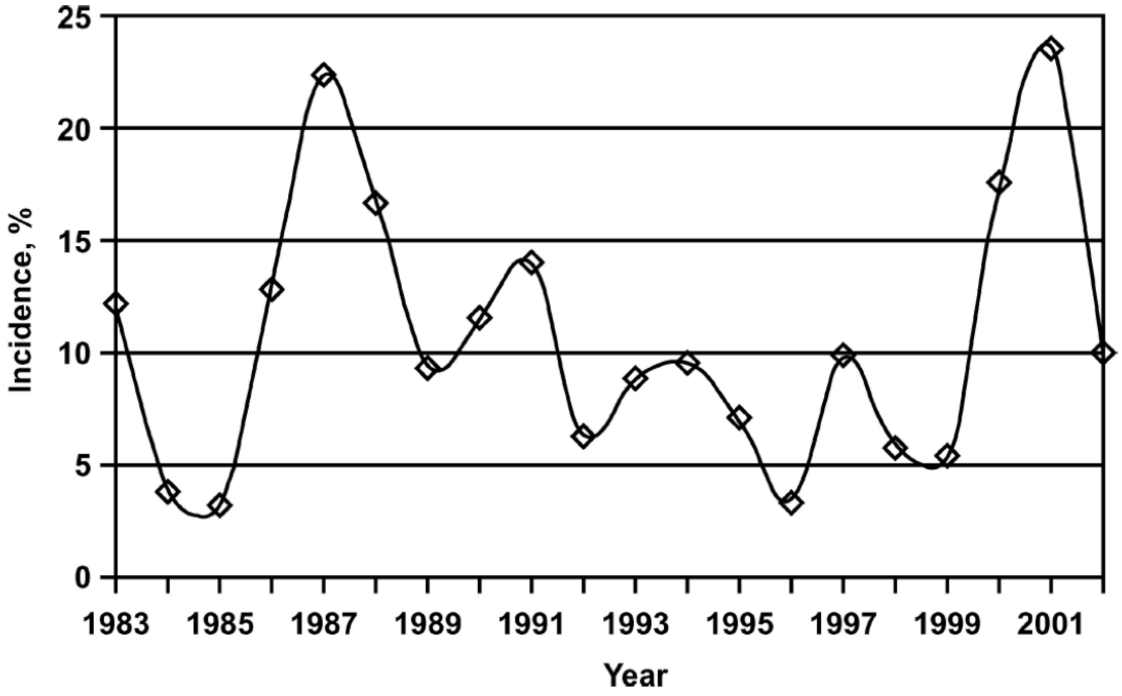
Introduction and Epidemiology

- **Most common and costly disease of beef cattle**
  - Nursing calves (Pre-weaning)
    - Variable levels of morbidity
      - Farm to farm
      - Year to year
  - Stocker and feedlot placements (Post-weaning)
    - 75% overall morbidity and mortality in feedlot placements
    - 90% overall morbidity and mortality in stocker populations

BOVINE RESPIRATORY DISEASE

Introduction and Epidemiology

- **Nursing Beef Calves – USDA MARC**



Snowder et al, 2005

BOVINE RESPIRATORY DISEASE

Introduction and Epidemiology

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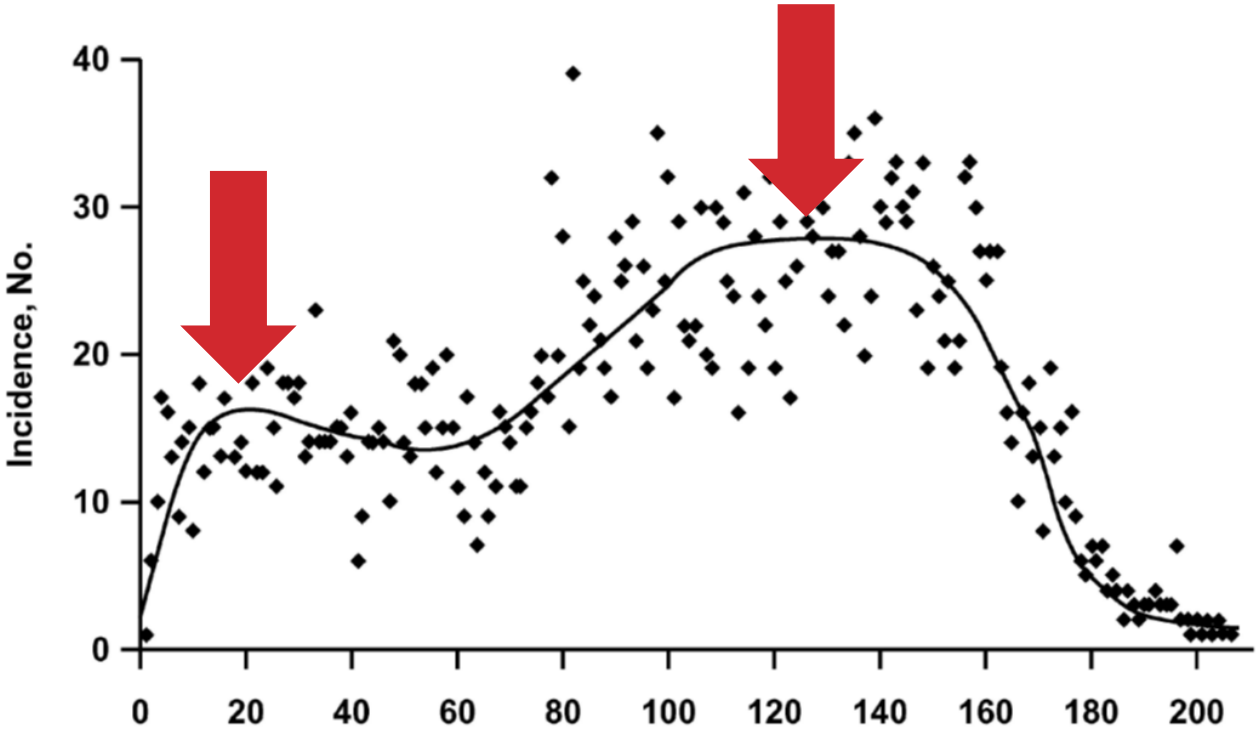


Snowder et al, 2005

BOVINE RESPIRATORY DISEASE

Introduction and Epidemiology

- **Nursing Beef Calves**

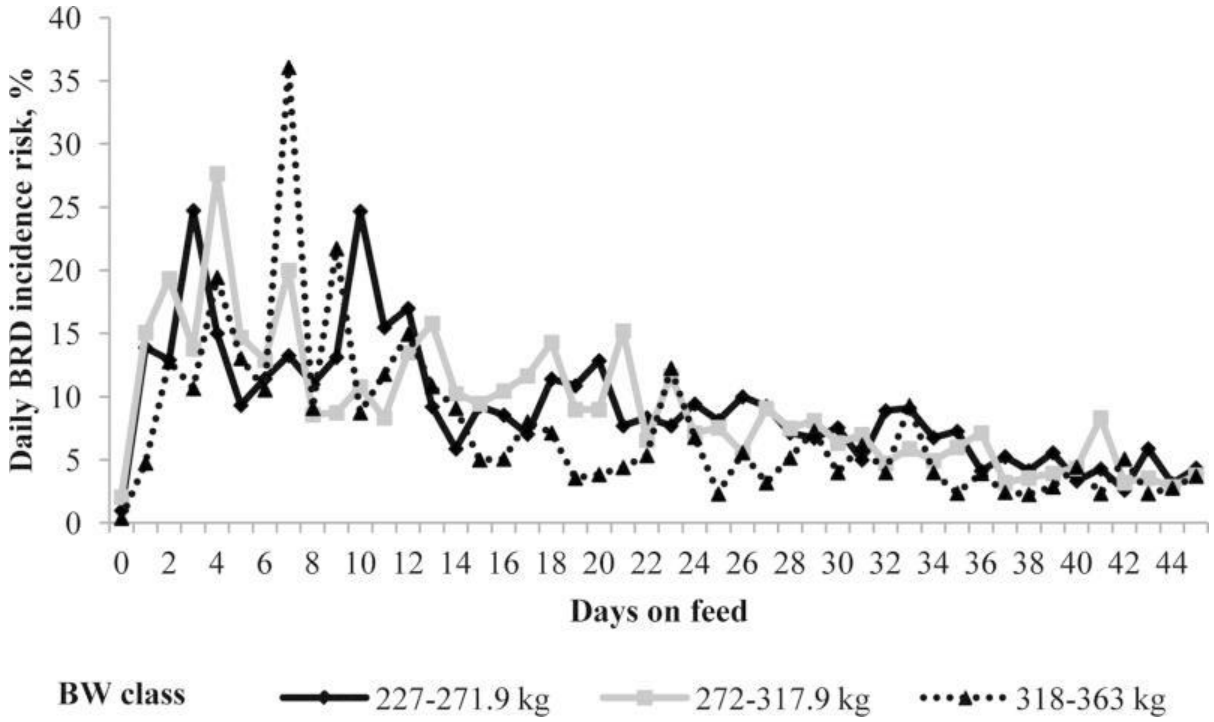
Pathogen	Percent of respondents identifying this pathogen
Mannheimia haemolytica	60
Pasteurella multocida	53
Mycoplasma bovis	37
Bovine respiratory syncytial virus (BRSV)	33
Bovine viral diarrhea virus (BVDV)	33
Infectious bovine rhinotracheitis virus (IBRV)	25
Histophilus somni	23
Coronavirus	12
Bibersteinia trehalosi	5
Parainfluenza type 3 virus (PI3V)	2
Other*	2

Woolums et al, 2014

BOVINE RESPIRATORY DISEASE

Introduction and Epidemiology

- **Stocker/Feedlot**



Cernicchiaro et al, 2012

BOVINE RESPIRATORY DISEASE

Introduction and Epidemiology

- **Stocker/Feedlot - Pathogens**
  - Fulton et al, 2009
    - 237 fatal cases of BRD observed from 2002 to 2003 in one Oklahoma feedyard
      - *M haemolytica* – 25%
      - *P multocida* – 25%
      - *Mycoplasma* spp – 71%
      - BRSV – 4.6%
      - Coronavirus – 11%

BOVINE RESPIRATORY DISEASE

Introduction and Epidemiology

- **Stocker/Feedlot - Pathogens**

Respiratory agent	Overall percent (proportion) positive	Animal-level prevalence (95% Confidence Intervals)	Prevalence by production class (95% Confidence Intervals)			
			Cow-calf	Dairy	Feedlot	Stocker
BRSV	3.81% (122/3205)	2.88% (1.9-4.4)	0.69% (0.1-3.5)	3.89% (2.2-6.8)	1.92% (0.6-5.8)	3.22% (1.3-7.6)
BHV-1	1.59% (51/3205)	1.59% (1.2-2.1)	1.49% (0.3-7.6)	1.45% (0.6-3.3)	0.53% (0.1-3.1)	0.47% (0.1-2.4)
BVDV	3.56% (114/3205)	3.92% (2.8-5.5)	1.21% (3.4-4.1)	3.94% (2.4-6.3)	4.98% (2.4-10.2)	5.06% (2.6-9.6)
IVD	8.30% (266/3205)	5.55% (3.4-9.0)	2.20% (0.5-9.5)	4.90% (2.4-9.6)	9.91% (3.2-26.9)	6.21% (2.1-17.0)
BCoV	43.81% (1404/3205)	36.05% (26.3-47.2)	15.98% (5.0-40.6)	32.75% (21.4-46.5)	24.96% (10.1-49.8)	69.51% (46.0-85.9)
M. bovis	20.12% (645/3205)	16.73% (11.4-24.0)	11.09% (3.1-33.1)	16.51% (9.7-26.7)	23.69% (9.4-48.1)	15.03% (5.9-33.2)
Multiple positive	17.32% (555/3205)	15.39% (11.3-20.7)	9.65% (3.4-24.5)	15.41% (10.0-23.0)	15.64% (7.1-31.2)	18.27% (9.2-33.0)

Lubbers et al, 2017

DIAGNOSTIC TESTS

DIAGNOSTIC TESTS

Two Important Characteristics

- **Sensitivity**
  - Probability of an animal being test positive given that it is truly diseased
    - **Freedom from false negatives**

- **Specificity**
  - Probability of an animal testing negative given that it is truly healthy
    - **Freedom from false positives**

DIAGNOSTIC TESTS

Sensitivity and Specificity

- **Determined by comparing test results to the results of a gold standard**

	TEST (+)	**TEST (-)**
DISEASE (+)	true positive	false negative
DISEASE (-)	false positive	true negative

DIAGNOSTIC TESTS

Sensitivity and Specificity

- **Characteristics of a test *per se***
  - Most useful when comparing performance of one test to another

	Sensitivity	Specificity	Accuracy
Abaxis CHAT™	78 (72–84)	97 (84–100)	81 (71–82)
IDEXX SNAP® Heartworm RT Test	84 (78–89)	97 (84–100)	86 (81–90)
Heska Solo Step®	79 (73–85)	97 (84–100)	82 (71–82)

DIAGNOSTIC TESTS

Positive and Negative Predictive Value

- **PPV = Probability that an animal that tests positive truly has the disease**

- **NPV = Probability that an animal that tests negative is truly healthy**

  - **PPV highly influence by prevalence**
    - **High prevalence = High PPV**
    - **Low prevalence = Low PPV**

DIAGNOSTIC TESTS

Population = 1000 high risk, auction market derived beef calves

Ear notched to determine BVD PI status

- **Sensitivity of ear notch = 99%**
- **Specificity of ear notch = 99%**
- **Prevalence of BVD PI = 0.4%**

DIAGNOSTIC TESTS

	TEST (+)	TEST (-)
DISEASE (+)	4	0
DISEASE (-)	11	985

PPV = 27%, NPV = 100%

DIAGNOSTIC TESTS

Population = 1000 high risk, auction market derived beef calves

Ear notched to determine BVD PI status

- **Sensitivity of ear notch = 99%**
- **Specificity of ear notch = 99%**
- **Prevalence of BVD PI = 2%**

DIAGNOSTIC TESTS

	TEST (+)	TEST (-)
DISEASE (+)	20	0
DISEASE (-)	11	969

PPV = 65%, NPV = 100%

DIAGNOSTIC TESTS

Agreement

- **How well the results of one test "agree" to results of another that tests the same quantity or parameter**
  - Cheaper test
  - Less invasive
  - Technically less challenging
- **Can we trust the results of our new test?**

DIAGNOSTIC TESTS

Agreement

- **Kappa coefficient**

Kappa Value	Interpretation
< 0.20	Poor Agreement
0.20 – 0.40	Fair Agreement
0.40 – 0.60	Moderate Agreement
0.60 – 0.80	Good/Substantial Agreement
0.80 – 1.00	Very Good/Almost Perfect Agreement

DIAGNOSTIC TESTS

Agreement

- **Agreement of NS, NPS, and BAL relative to TTW in dairy calves with BRD**

Pathogen	[a]Method	[b]No. Calves with Each Combination of Results +/+	+/−	−/+	−/−	Percent Positive Agreement	Kappa (95% CI)	[c]*P*
Mannheimia haemolytica	NS	16	0	4	80	88.9 (78.1, 99.7)	0.86 (0.74, 0.99)	.125
	NPS	15	1	2	82	90.9 (80.7, 100)	0.89 (0.77, 1.00)	1.00
	BAL	15	1	2	82	90.9 (80.7, 100)	0.89 (0.77, 1.00)	1.00
Pasteurella multocida	NS	57	2	3	38	95.8 (92.1, 99.5)	0.90 (0.81, 0.99)	1.00
	NPS	58	1	3	38	96.7 (93.4, 99.9)	0.92 (0.84, 1.00)	.625
	BAL	57	2	3	38	95.8 (92.1, 99.5)	0.90 (0.81, 0.99)	1.00
Mycoplasma spp.	NS	81	3	5	0	95.3 (92.0, 98.6)	−0.04 (−0.10, 0.01)	.727
	NPS	78	8	5	0	92.3 (88.1, 96.5)	−0.07 (−0.14, −0.01)	.581
	BAL	81	1	5	0	96.4 (93.6, 99.3)	−0.02 (−0.06, 0.02)	.219
Mycoplasma bovis	NS	41	7	1	39	91.1 (85.0, 97.3)	0.82 (0.70, 0.94)	.070
	NPS	41	4	3	35	92.1 (86.3, 97.9)	0.83 (0.71, 0.95)	1.00
	BAL	45	1	2	40	96.8 (93.1, 100)	0.93 (0.86, 1.00)	1.00
Bovine respiratory syncytial virus	NS	7	7	2	78	60.9 (37.3, 84.4)	0.56 (0.30, 0.81)	.180
	NPS	11	4	2	76	78.6 (61.8, 95.3)	0.75 (0.56, 0.94)	.688
	BAL	13	0	2	78	92.9 (83.0, 100)	0.92 (0.80, 1.00)	.500
Bovine coronavirus	NS	6	0	7	76	63.2 (37.8, 88.5)	0.59 (0.33, 0.86)	.016
	NPS	6	0	9	70	57.1 (31.9, 82.4)	0.52 (0.27, 0.78)	.004
	BAL	6	0	3	77	80.0 (57.8, 100)	0.78 (0.55, 1.00)	.250

Doyle et al, 2017

DIAGNOSTICS FOR BRD

DIAGNOSTICS FOR BRD

Rationale for The Use of Diagnostic Tests

- **BRD = Multifactorial**
  - Colonization and infection of the LRT with viral and bacterial pathogens
- **Monitoring for specific pathogens might be useful**
  - Design of preventive health programs
  - Prevalence of AMR in bacterial pathogens
    - Therapeutic regimens

DIAGNOSTICS FOR BRD

When Should Diagnostics Be Considered?

- **Not necessary for every animal on every operation**
  - Design of treatment/control regimens on individual operations
    - Prevalence of specific pathogens
    - Antimicrobial susceptibilities
  - Recurrent outbreaks within a population
    - Unusual patterns of disease within an outbreak
    - High morbidity/mortality
  - Lack of response to symptomatic therapy

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Gold standard = analysis of tissue from lung**
  - Post-mortem
  - Biased representation of disease process
    - End-stage of disease
      - Cause of death rather than cause of disease
    - Treatment failures
      - Multiple treatments given
      - Over-representation of antimicrobial resistant bacteria

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Lung biopsy**
  - Antemortem collection of lung tissue
    - Fewer issues with airway contaminants
  - Acute BRD prior to therapy
    - Better representation of cause of disease?
      - Pathogens
      - Antimicrobial susceptibility

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Lung biopsy**
  - Burgess et al, 2011
    - Samples collected from right cranioventral lung (ICS 2)
      - Few side effects
      - Technically challenging
      - 43% success in field settings
      - Poor agreement with lung culture

DIAGNOSTICS FOR BRD

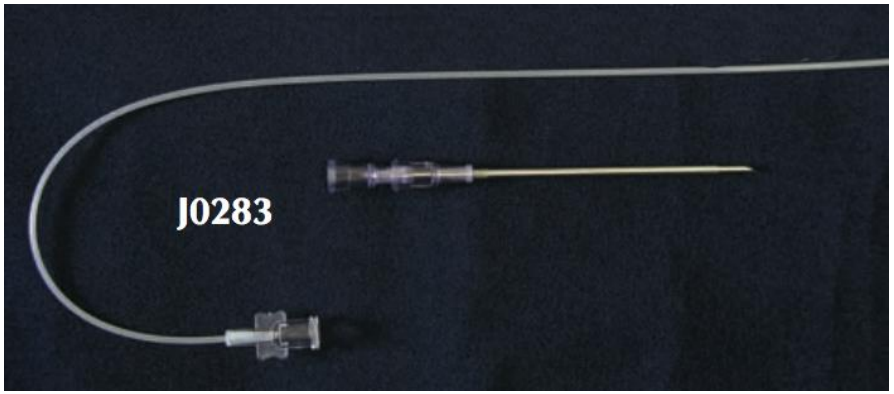
What Diagnostics Should I Choose?

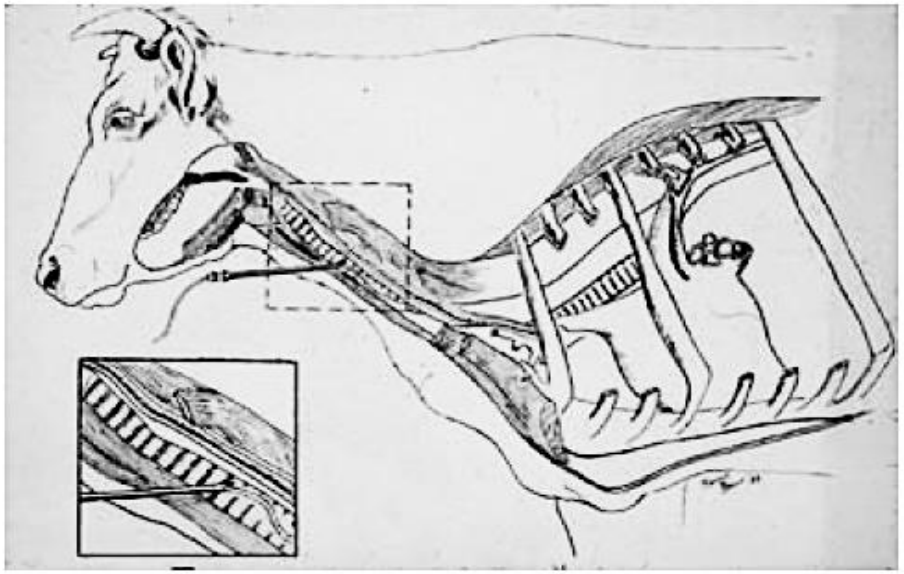
- **Other available tests**
  - Transtracheal wash (TTW)
  - Bronchoalveolar lavage (BAL)
  - Deep Nasopharyngeal Swab (DNP)
  - Nasal Swab (NS)

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **TTW**
  - Percutaneous passage of sterile tubing through trachea for collection of pooled pulmonary secretions at tracheal bifurcation





DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **BAL**
  - Passage of sterile tubing through nares and into the lower airway (guided or unguided) and wedged in a bronchus
  - Sterile fluid infused (120-240 ml), aspirated

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **DNP**
  - Passage of a guarded catheter through nares and into the nasopharynx

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Nasal Swab**
  - Passage of sterile culture swab into external nares

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Each test = advantages and disadvantages**
  - Cost
  - Technical difficulty
  - Area of respiratory tract that is sampled
- **How do the available tests AGREE with one another relative to an established reference method?**
  - How can I incorporate this into my practice?

DIAGNOSTICS FOR BRD

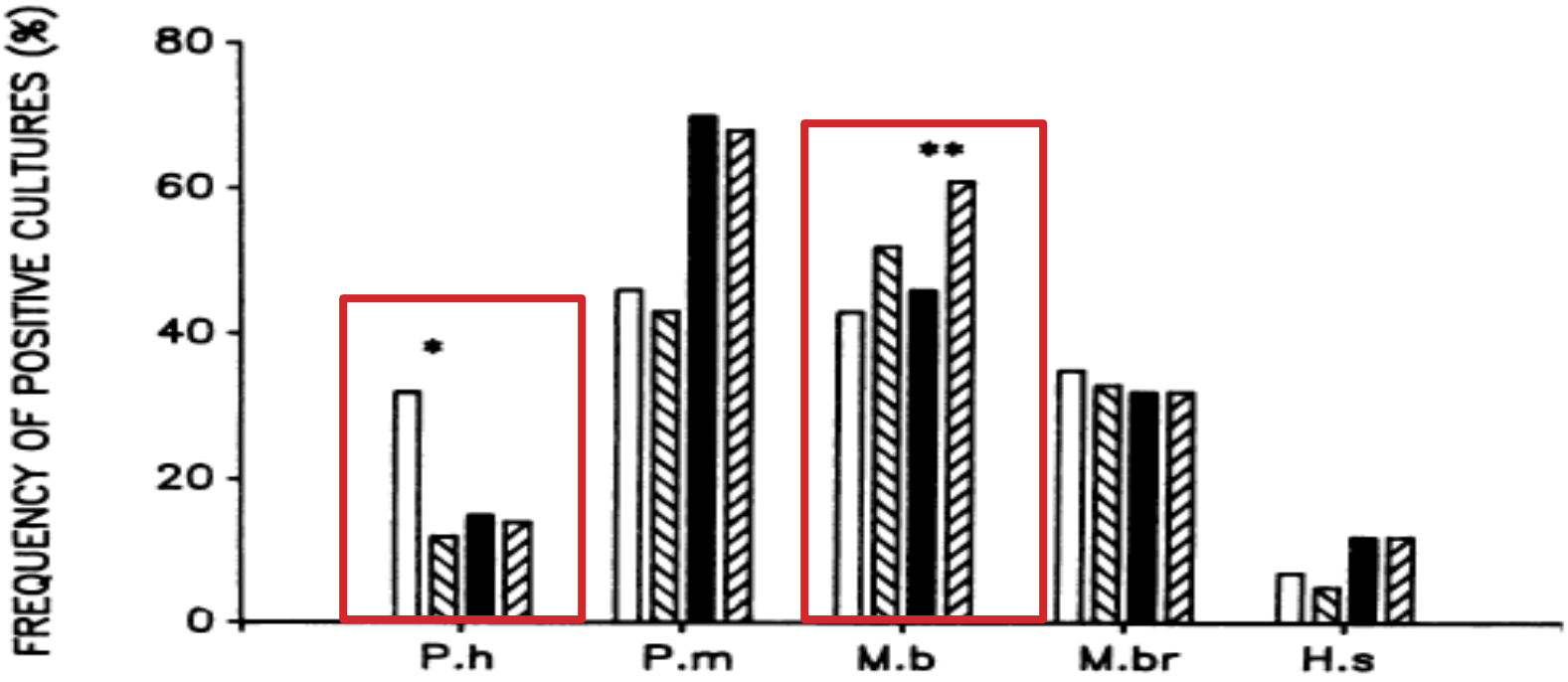
What Diagnostics Should I Choose?

- **Allen et al, 1991**
  - Comparison of DNP and BAL in feedlot calves with naturally occurring BRD
    - Agreement between DNP and BAL moderate for most pathogens at individual calf level
      - *M. haemolytica* = 0.47
      - *P. multocida* = 0.61
      - *M. bovis* = 0.53
      - *H. somni* = 0.55

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Allen et al, 1991**



Group Level Agreement Between DNP and BAL

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **DeRosa et al, 2000**
  - Comparison of DNP to guarded TTW
    - 45 beef calves with acute BRD sampled once at diagnosis
      - DNP isolates genetically identical to TTW samples 70% of time
      - If both methods positive: same organism present 96% of the time
      - Antimicrobial susceptibilities for sampling methods identical
    - Conclusion: DNP comparable to guarded TTW

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Godinho et al, 2007**
  - Comparison of DNP to post-mortem lung lavage
    - 4-6 month old beef calves with naturally occurring BRD
      - PPV = 100% (*M. haemolytica* and *M. bovis*)
      - NPV = 67% (*M. haemolytica*) and 33% (*M. bovis*)
      - Susceptibility to tulathromycin equivalent for all samples for both methods
  - Conclusion: NPS comparable to lung lavage

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Doyle et al, 2017**
  - Agreement between 4 sampling methods in dairy calves with acute BRD
    - Dairy calves > 30 days of age with acute BRD
      - Agreement of NS, DNP, and BAL relative to TTW good for identification of:
        - *M. haemolytica*
        - *P. multocida*
        - *M. bovis*

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Doyle et al, 2017**

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Agreement of NS, NPS, and BAL relative to TTW in dairy calves with BRD

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

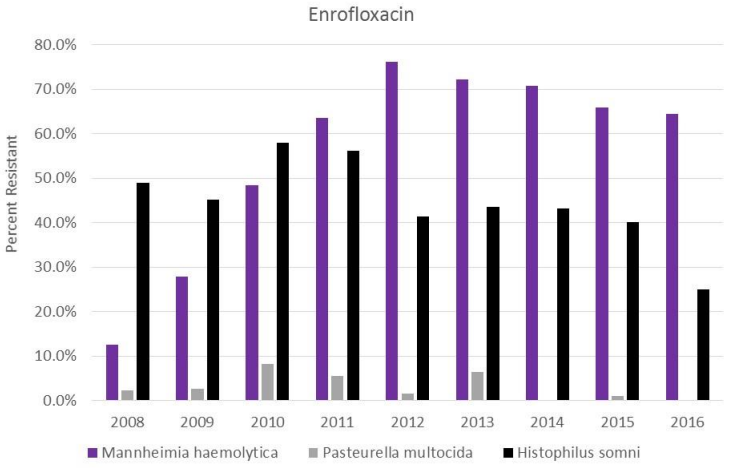
- **Capik et al, 2017**
  - Comparison of DNP to BAL obtained from beef calves with BRD
    - 28 mixed-breed beef calves 5 days after diagnosis of naturally occurring BRD
      - *M. haemolytica*
        - PPV and NPV of DNP = 67% and 100%
          - Kappa = 0.73
      - *P. multocida*
        - PPV and NPV of DNP = 75% and 100%
          - Kappa = 0.81
      - *H. somni*
        - PPV and NPV of DNP = 100% and 96%
          - Kappa = 0.78

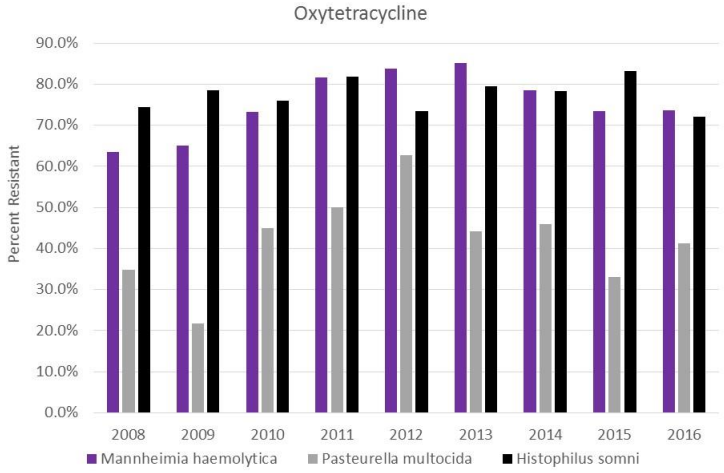
DIAGNOSTICS FOR BRD

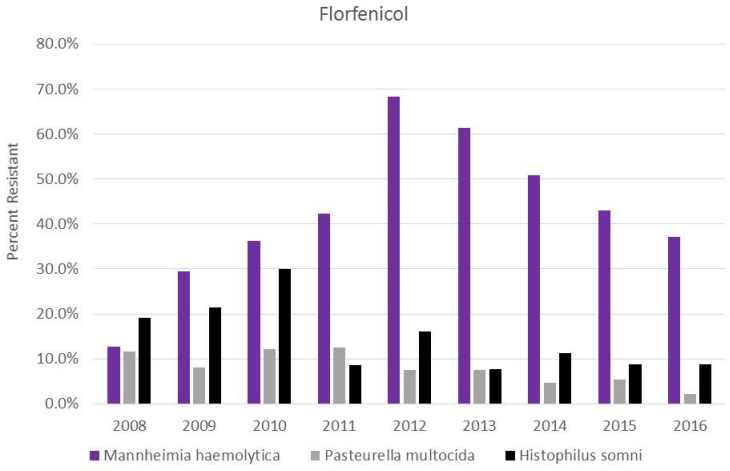
What Diagnostics Should I Choose?

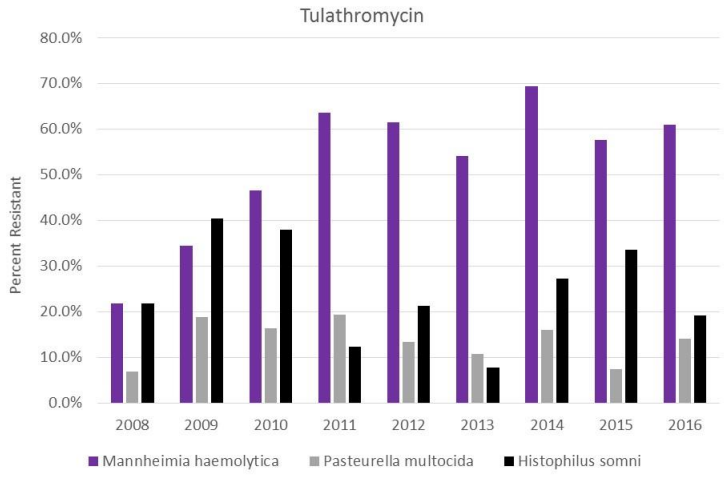
- **Methods of Pathogen Detection with Testing Methods**
  - For bacteria
    - Culture
      - Preferred for *M. haemolytica*, *P. multocida*, *H. somni*
        - Antimicrobial susceptibility
    - PCR
      - Preferred for *M. bovis (*from characteristic lesion)
  - For viruses
    - PCR
      - Enhanced sensitivity/specificity with faster turnaround
      - Multiplex options available

DIAGNOSTICS FOR BRD



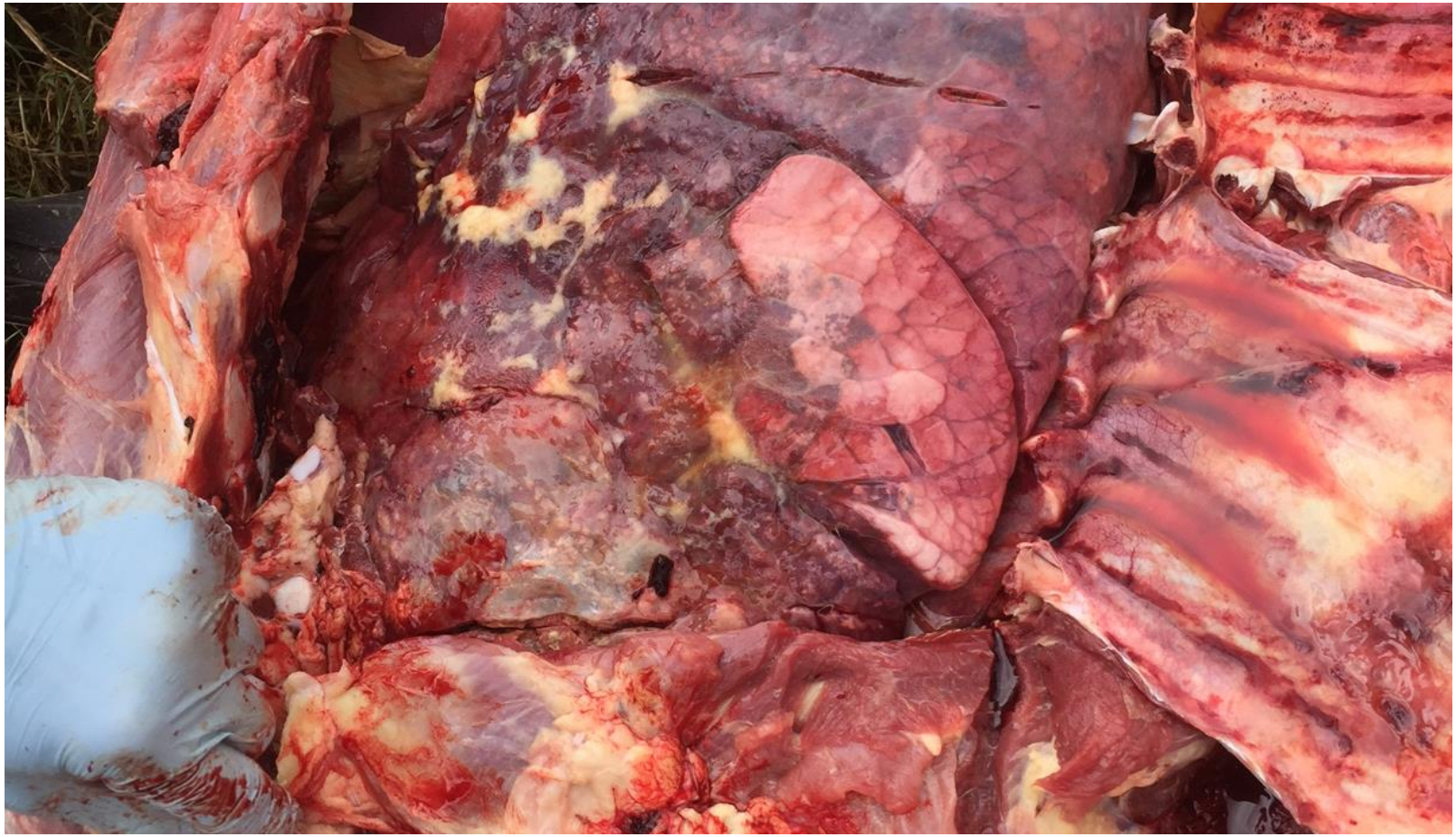






Resistance to Antimicrobials in BRD Pathogens
Courtesy of Brian Lubbers, K State

DIAGNOSTICS FOR BRD



DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Methods of Pathogen Detection with Testing Methods**
  - Viral PCR
    - Potential to detect vaccine virus in animals recently vaccinated with MLV vaccines
      - Parenteral and intranasal
        - BHV-1
        - BVDV
      - Shed for up to 3 weeks

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Take Home Points**
  - No perfect antemortem test exists and each sampling method has advantages and disadvantages
  - Choice of tests depends on multiple factors
    - Individual vs Population
    - Specific pathogens of concern
      - Coronavirus
      - BRSV

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Take Home Points**
  - *In an individual calf*
    - BAL or TTW preferred over DNP or NS
  - *In a population of cattle*
    - DNP (or NS) will provide results that can be interpreted with some level of confidence that findings are real
  - *For Coronavirus*
    - DNP (or NS) preferred to BAL or TTW
  - *For BRSV*
    - BAL or TTW preferred to DNP or NS

DIAGNOSTICS FOR BRD

What Diagnostics Should I Choose?

- **Take Home Points**
  - Culture preferred for most bacterial pathogens
    - Antimicrobial susceptibility
  - PCR probably preferred for *M. bovis* and viral pathogens
    - Faster turnaround with multiplex options
    - Need to understand benchmarks for shedding within a specific population
      - Coronavirus
    - Consideration of vaccination history essential
      - BHV-1 and BVDV

QUESTIONS